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Isolation and characterization of tamarind kernel gum: A novel natural floating polymer

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ABSTRACT

Floating drug delivery systems (FDDS) are aimed to retain the drug in the stomach and are useful for drugs that are poorly soluble or unstable in intestinal fluids. The underlying principle is very simple i.e., to make the dosage form less dense than the gastric fluids so that it can float on them. The drug usually keeps floating in the gastric fluid and slowly dissolves at a pre-determined rate to release the drug from the dosage form and maintain constant drug levels in the blood. Several approaches are currently used to retain the dosage form in the stomach. The principle of the floating tablets is simple and practically approach to achieve increased gastric residence time to obtain controlled release. The polymers play an important role in the formulation of floating drug delivery systems as they should make the dosage form less dense and able to release the drug from the dosage form in a controlled manner. Though, there are several floating polymers available in the market, there is continuous need to develop floating polymers which are safe and inexpensive. The aim of the work was to isolate and characterize the Tamarind kernel gum as novel floating polymer.

Keywords: Floating Polymer, Controlled release, Tamarind Kernel Gum.

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INTRODUCTION

Seeds gums are important agrochemicals used in various industries worldwide. The growing industrial utility of these gums in the field of paper, textile, petroleum recovery and pharmaceutical industries has resulted in an impetus in India for intensified research on new sources of gums and their modified products.

Gum is a by-product obtained as a result of metabolic mechanism of plants. Natural gums are either water soluble or absorb water to form a viscous solution[1]. Natural gums are economic, easily available. Gums have been wildly used as tablet binders, emulgents and thickeners in cosmetics and suspensions as film-forming agents and transitionalcolloids[2].

Polysaccharides are the choice of materials among the hydrophilic polymers used, because they are nontoxic and acceptable by regulating authorities[3]. The various polysaccharides used in drug delivery application are cellulose ethers[4], xanthan gum[5], locust bean gum[6] and guar gum[7]. Another natural gum *Tamarind kernel* gum obtained from the seed kernel of *Tamarindus indica,* possesses properties like high viscosity, broad pH tolerance, non-carcinogenicity, mucoadhesive nature and biocompatibility[8]. It is used as stabiliser, thickener, gelling agent, and binder in food and pharmaceutical industries. The tamarind seed polysaccharide constitutes about 65% of the tamarind seed components[9]. It is a branched polysaccharide with a main chain of β-d-(1,4)- linked glucopyranosyl units, and that a side chain consisting of single d- xylopyrnosyl unit attached to every second, third, and fourth d-glucopyranosyl units through an β-d-(1,6) linkage. One d-galactopyranosyl unit is attached to one of the xylopyrnosyl units through a β-d-(1, 2) linkage[10]. In the present study an effort was made to extract the tamarind gum from the tamarind seeds and to evaluate their physical characteristics.

MATRIALS AND METHODS

Isolation of Gum from Tamarind Seeds:

The crushed seeds of Tamarindus Indica were soaked in water for 24 h, boiled for 1h and kept for 2h for the release of gum into water. The soaked seeds were taken and squeezed in a muslin bag to remove marc from the filtrate. Then, to the

filtrate, equal quantity of acetone was added to precipitate the gum. The gum was separated by filtration. The marc was not discarded but it was sent for multiple extractions with decreasing quantity of extracting solvent, i e., water with the increase of number of extractions. The isolation was continued until the material was free of gum. The separated gum was dried in hot air oven at temperature 40^0 C. The dried gum was powdered and stored in air tight containers at room temperature.

Fig 1: (a) Photograph Showing Plant Part Selected For Study

Fig 1: (b) Photograph Showing Extracted *Tamarind Kernel* Gum

Phytochemical Examination: [11-12]

For the detection of the presence of carbohydrates, reducing sugars, tannins, mucilage and peroxide enzymes the standard tests Molisch's test for carbohydrate, reduction of Fehling's solution for reducing sugars, ferric chloride test for tannins, ruthenium red test for *tamarind kernel* gum were done.

1. **Test For Carbohydrates** (With aqueous test solution):

Molisch's Test[16]**:**

To the aqueous solution of *Tamarind Kernel* gum, few drops of alcoholic α-naphthol were added and to it few drops of concentrated sulphuric acid was added through sides of the test tube.

2. **Test For Proteins:**

Ninhydrine Test[17]**:**

To the aqueous solution of *Tamarind Kernel* gum, ninhydrine solution was added and then this solution was boiled.

3. **Test For Alkaloids:**

Wagner's Test[18]**:**

To the aqueous solution of *Tamarind Kernel* gum, Wagner's reagent was added.

4. **Test For Tannins:**

Ferric Chloride Test[19]**:**The extract was treated with ferric chloride solution.

5. Confirmatory Test for Chlorides: (Silver Nitrate Test) [20]**:**

Small amount of sodium extract was taken in a semi micro test tube and it was neutralised with dilute nitric acid and then silver nitrate was added.

6. Test for Sulphates[21]**:**

Small amount of sodium carbonate extract was taken in a semi micro test tube and it was neutralised with dilute nitric acid. To this solution 5 drops of Barium chloride solution was added finally.

7. Fehling's Test[22]**:**

To the aqueous solution of *Tamarind Kernel* gum, few drops of Fehling's reagent was added.

8. Determination of Sulphated Ash[23]**:**

A platinum dish was heated to redness for 10 minutes and it was allowed to cool in a dessicator and its weight was noted. 1 g of substance was placed and examined in a dish; it was moistened with sulphuric acid, ignited gently, again moistened with sulphuric acid and ignited at about 800^0C. Then it was cooled, weighed again, ignited for 15 minutes and this procedure was repeated until 2 successive weighings do not differ by more than 0.5mg.

9. Test For Arsenic[24]**:**

5 ml of the test solution was heated on a water-bath with as equal volume of hypo phosphorous agent.

10. Test For Mucilage:

Ruthenium red test[25]**:**

To the aqueous test solution, little amount of ruthenium red solution was added.

11. Determination of Loss on Drying[26]**:**

1 gm of the gum was transferred into a Petri dish and then dried in an oven at 105 ± 5 ºC until a constant weight of gum was obtained. The moisture content was then determined as the ratio of moisture loss to weight of sample expressed as a percentage. Loss on drying is loss of weight expressed as %w/w.

12. Test for Foreign Matter[27]**:**

100 – 500 mg of substance to be examined was weighed and was spreaded out in a thin layer. Foreign matter was examined by inspection with unaided eye or by use of a lens (6x). Separate foreign matter was separated & weigh & the percentage present in it was calculated.

13. Determination of Ash Value[27]:

1gm of gum was accurately weighed and evenly distributed it in the crucible. It was dried at 105ºC for one hour and ignited in muffle furnace at 600 ± 25 ºC. Ash content was estimated by the measurement of the residue left after the combustion in the furnace.

14. Determination of Acid Insoluble Ash[27]:

The ash was boiled for 5 min with 25 ml of 2 M HCl; the insoluble matter was collected in a sintered glass crucible or on an ash less filter paper, washed with hot water & ignited. The percentage of acid insoluble ash was calculated with reference to air dried drug.

15. Determination of Water Soluble Ash[27]:

The ash, obtained during the determination of ash value, was boiled for 5 min with 25 mL of water. The insoluble matter was collected on ash less filter paper and washed with hot water. The insoluble ash was then transferred into silica crucible, ignited for 15 min, and weighed. The procedure was repeated to get a constant weight. The weight of insoluble matter was subtracted from the weight of the total ash. The difference of weight was considered as water-soluble ash. The percentage of water-soluble ash was calculated with reference to the air dried sample.

Physicochemical Characterization of Mucilage:

The separated *tamarind kernel* gum was evaluated for solubility, swelling index, loss on drying, ash value, density, compressibility index and angle of repose.

I. Angle of Repose:

The frictional force in a loose powder can be measured by 'Angle of Repose' (θ). It is defined as the maximum angle possible between the surface of the pile of the powder and the horizontal plane [28]. If more powder is added to the pile, it slides down the sides of the pile until the mutual friction of the particles producing a surface angle, is in equilibrium with the gravitational force

The angle of repose of gum was determined by the fixed funnel method. A funnel was secured with its tip at a given height (h), above a graph paper that is placed on a flat horizontal surface. The accurately weighed (10gms) gum was taken and was allowed to flow freely through the funnel on to the surface until the apex of the conical pile just touches the tip of the funnel. The radius of the formed cone was measured and angle of repose (θ) was calculated using the following equation:

$$\theta = \tan^{-1}\frac{h}{r} \quad \text{(or)} \quad \tan\theta = \frac{h}{r}$$

Where, θ = angle of repose; h = height of the cone; and r = radius of the cone base.

Table 1: Correlation between Angle of Repose and the Flow Property of Powders

Angle of Repose	Flow Property
< 25	Excellent
25-30	Good
30-40	Passable
> 40	Poor

II. Bulk Density:

Density is defined as weight per unit volume. Bulk density (D_b) was determined by measuring the volume (V_b) of known weighed quantity (W) of powdered gum using bulk density apparatus and can be calculated by using the formula:

$$\text{Bulk Density } (D_b) = \frac{\text{Mass of Powder (W)}}{\text{Bulk Volume of Powder } (V_b)}$$

The bulk density of a powder primarily depends on the particle size distribution, particle shape and the tendency of particles to adhere together [28].

III. Tapped Density:

Tapped density (D_t) was determined by measuring the volume (V_t) of known weighed quantity (W) of powdered gum after desired mechanical tapping using tapped density tester which provides a fixed drop of 14 ± 2 mm at a nominal rate of 300 drops per minute. The cylinder was tapped 500 times initially followed by an additional tap of 750 times until difference between succeeding measurement is less than 2% and then the Tapped Volume, V_t was measured, to the nearest graduated unit. The tapped density can be calculated by using the formula [28]:

$$\text{Tapped density } (D_t) = \frac{\text{Mass of powder (W)}}{\text{Tapped volume of powder } (V_t)}$$

IV. Hausner's Ratio:

The Hausner's ratio was obtained by dividing the tapped density by the bulk density of the gum powder. Lower the value of Hausner's ratio better is the flow property (Table 2).

$$\text{Hausner's ratio} = \frac{\text{Tapped density of powder } (D_t)}{\text{Bulk density of powder } (D_b)}$$

Table 2: Correlation between Hausner's Ratio and the Flow Property of Powders

Hausner's Ratio	Flow Property
< 1.18	Excellent
1.19 – 1.25	Good
1.3 – 1.5	Fair to Passable
> 1.5	Poor
Above 2	Extremely Poor

V. Carr's Index:

The Carr's index (% compressibility) of the gum powder was calculated from the difference between the tapped and bulk densities divided by the tapped density and the ratio is expressed as a percentage [28].

$$\text{Carr's index (\%)} = \frac{\text{Tapped density } (D_t) - \text{Poured density } (D_b)}{\text{Tapped density } (D_t)} \times 100$$

VI. Swelling Index (SI):

About 1g of gum powder was placed in 100 ml stoppered measuring cylinder and the initial volume occupied by the gum was noted. The volume was made up to 100 ml mark with distilled water. The contents were mixed gently for 2 minutes and set aside for 24 hours. Then the supernatant was carefully decanted and the volume of sediment was

measured. The Swelling Index was compute using the equation:

$$S = \frac{V_2}{V_1}$$

Where, S = Swelling Index

V_1 = Volume occupied by the gum prior to hydration

V_2 = Volume occupied by the gum after hydration [28].

Determination of Viscosity:

1 g of dried and finely powdered gum was suspended in 75 ml of distilled water for 5 h. Distilled water was added up to 100 ml to produce the concentration of 1 % w/v. The mixture was homogenized by mechanical stirrer for 2 h and its viscosity was determined using a Brookefield viscometer, spindle-LV2 (Brookefield LV-II, USA) at 20 rpm and 25^0 C.

Characterization of *Tamarind Kernel* Gum

X-ray Diffraction:

Diffraction pattern of powdered Tamarind seeds powder was recorded with an X-ray diffractometer (Panalytical species Pvt.Ltd, Singapore), X-ray diffraction was performed at room temperature (30^0 C) with a diffractometer; target, Cu(λ=1.54A^0), filter, Ni; Voltage, 40 KV; current 30mA; time constant 10mm/s; scanning rate 2^6/min; measured from 10-350 at full scale of 200. The X-ray Diffraction of *Tamarind Kernel* gum is shown in Fig 3.

Fourier Transform Infrared (FTIR) Spectroscopy

FTIR spectra of Tamarind seeds powder were recorded on samples prepared in potassium bromide (KBr) disks using Shimadzu Corporation, (Tokyo, Japan) Model-1601 PC. Samples were prepared in KBr disks by means of a hydrostatic press at 6-8 tons pressure. The scanning range was 500 to 4000 cm^{-1}. The FTIR spectra of *Tamarind Kernel* gum is shown in Figure 3 and the peaks shown at different wave lengths are mentioned in Table 5.

In the spectroscopy, infrared radiation is absorbed by the sample and some of it is transmitted. The resulting spectrum represents the molecular absorption and transmission, creating a molecular fingerprint of the sample. Like a fingerprint, no two unique molecular structures produce the same infrared spectrum.

RESULTS AND DISCUSSION

Physiochemical Characterization of *Tamarind kernel* gum

Polysaccharide gum derived from the seeds of tamarind, *Tamarindus indica* was an off white to cream colour powder, and the viscosity of its 1% aqueous dispersion was 450 cP indicate that the gum is colloidal in nature following non-Newtonian bodies which do not settle down quickly. The gum obtained was subjected to physiochemical characteristics the results of which are summarised in Table 3. The swelling ratio of the gum makes the polymer to control the release of drug.

Table 3: Physiochemical Characterization of *Tamarind kernel* gum

Parameters	Observation
Solubility	Slightly soluble in water. Practically insoluble in alcohol, chloroform and acetone.
pH (1% w/v solution)	5.5
Loss on drying	1.4%
Ash value	4.9%
Water soluble ash	3.8%
Acid insoluble ash	0.6%
Sulphated ash	2%
Test for foreign matter	Less than 0.1%
Test for Arsenic	Less than 1 ppm
Swelling ratio	
In water	12
In 0.1 N HCL	10
In phosphate Buffer 7.4	6.0
True density	0.80 g/cc
Bulk density	0.59 g/cc
Tapped density	0.79 g/cc
Compressibility index	15.03%
Hausner's ratio	0.2
Description Powder	Light brown coloured granular powder
Angle of repose	20.82

The angle of repose of the gum shows that it has excellent flow characteristics, which can be used in the formulation of floating dosage forms.

Phytochemical Characterization of *Tamarind kernel* gum

The Violet coloured ring appeared at the junction and this confirmed the presence of carbohydrates in the *Tamarind Kernel* Gum. No violet colour was formed indicating the absence of proteins in *Tamarind Kernel* gum. No reddish brown precipitate was formed with Wagner's reagent indicating the absence of alkaloids in *Tamarind Kernel* gum. No blue or green coloured appeared indicating the absence of tannins in *Tamarind Kernel* gum. No white precipitate was formed indicating the absence of chlorides in *Tamarind Kernel* gum. Formation of white precipitate insoluble in concentrated nitric acid is obtained indicating the presence of sulphates in *Tamarind Kernel* gum. Brick red precipitate of cuprous oxide was formed indicating the presence of reducing substances in *Tamarind Kernel* gum. No brown precipitate was obtained indicating the absence of arsenic. Pink colour appeared indicating the presence of mucilage in *Tamarind Kernel* gum.

There was no black precipitation for tannin with ferric chloride solution. The presence of mucilage was tested by treating the gum mucilage with ruthenium red solution and Benzidine solution, formation of pink colour with Ruthenium red and blue colour with Benzidine solution indicate the presence of mucilage. To know whether the gum contains the peroxidise enzymes, which is commonly present in some gums like gum acacia. It was treated with few drops of hydrogen peroxide, no blue colour formation; indicate the absence of enzymes in it. Thus a chance of oxidative degradation due to gum as excipient is eliminated as compared to gum acacia. Mucilage gum on treating with Ninhydrine reagent does not give purple colouration indicating the absence of amino acids. The results of Phytochemical screening of *tamarind kernel* gum are summarised in Table 4.

Table 4: Phytochemical Screening of *Tamarind Kernel* Gum

S.No	Tests	Observation
1.	Test for Carbohydrates (Molisch's test)	+
2.	Test for Tannins (Ferric chloride test)	–
3.	Test for proteins (Ninhydrine test)	–
4.	Test for alkaloids (Wagner's test)	–
5.	Test for glycosides (Keller-killaini test)	–
6.	Test for mucilage (Ruthenium red test)	+
7.	Test for flavonoids (Shinoda test)	–
8.	Test for reducing sugar (Fehling's test)	+
9.	Mounted in 95 % alcohol	Transparent angular masses under microscope.
10.	Mounting in the iodine	No blue coloured particles (starch absent)
11.	Test with cupric-tartaric solution	Red precipitate is produced.
12.	Warming with 5 M sodium hydroxide	A brown colour is produced.
13.	Test for chlorides (silver nitrate test)	-ve
14.	Test for sulphates (barium chloride test)	+ve

Characterization of *Tamarind Kernel* Gum:

X-ray Diffraction Analysis:

The X-ray diffraction pattern (Fig 3) of *Tamarind kernel* gum did not show any characteristic peak, which indicates that the structure is completely amorphous.

Fourier Transform Infrared (FTIR) Spectroscopy Analysis

The absence of sharp peak at 1700–1800 cm^{-1} in the FTIR spectrum indicates that there is no carboxyl group in the extracted sample. FTIR spectral analysis of *Tamarind Kernel* gum is shown in Table 3.

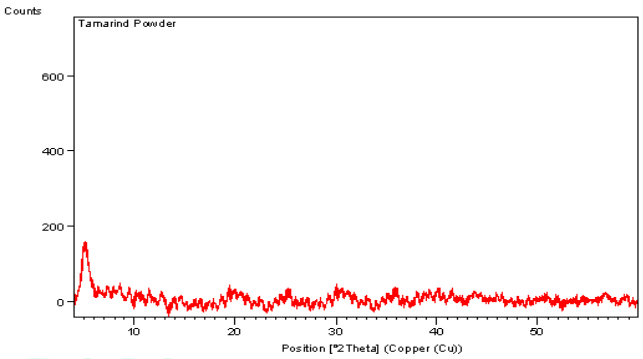

Figure 2: X-ray Diffraction Pattern of *Tamarind Kernel* Gum

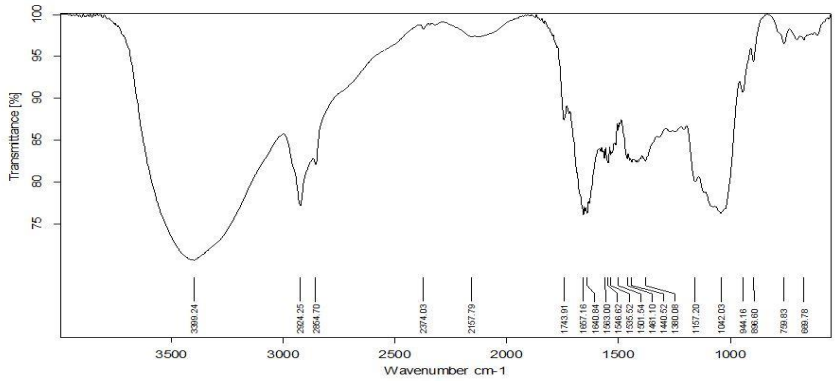

Figure 3: FTIR Spectra of *Tamarind Kernel* Gum

Table 5: FTIR Spectral Analysis of *Tamarind Kernel* Gum

Sample	Frequency of Peak	Functional Group
Tamarind Kernel gum	3399.24	Secondary OH
	2924.25	C-H Stretching
	1640.84	C=O Stretching
	1657.16	-CHO (aldehyde)
	1042.03	C-O-C

CONCLUSION

The result of the present study demonstrated that the *tamarind kernel* gum obtained from seed kernel of plant *Tamarindus indica* is light brown coloured granular powder which is amorphous in nature. It is soluble in glacial acetic acid, slightly soluble in water, practically insoluble in alcohol, chloroform and acetone and forms thick gel can control the drug release.

Moreover as this plant is widely distributed in nature, tamarind are eaten by the local tribes and used as food supplement, available chiefly in India and many other countries and easily available option without destroying the natural sources as compared to that of the other available natural option will be one of the suitable options to utilize as pharmaceutical controlled release floating polymer. Since the primary ingredients are inexpensive, devoid of toxicity, biocompatible, biodegradable, less dense and easy to manufacture, they can be used in place of currently marketed sustained release floating polymers.

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